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Inhibitor-2 *In Vivo* Enhances Protein Phosphatase-1 Activity and Suppresses Learning and Memory: Possible Implication for the Progression of Tau Pathology

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Review of Yang et al.

Learning and memory are important cognitive functions, vital to survival. Diseases impairing these functions thus have huge consequences for the normal functioning of an individual. Illnesses associated with dementia, such as Alzheimer's disease (AD), currently contribute significantly to the worldwide disease burden, with an estimated 47.5 million people living with dementia in 2015 (World Health Organization, 2015). As this number is predicted to double each year, it will bring extensive personal and societal costs. Unfortunately, the neurobiological mechanisms underlying deficits associated with learning and memory are still not fully understood. Studies addressing the molecular and cellular underpinnings of normal learning and memory will greatly improve our understanding of memory-related impairments and might point to much-needed therapeutic interventions.

One biological process related to learning and memory is protein phosphorylation and dephosphorylation. For example, protein phosphatase-1 (PP1) was found to attenuate memory in a mouse model (Genoux et al., 2002). PP1 is a major phosphatase of CREB (cAMP/calcium response element-binding protein), which promotes gene expression essential for memory formation (Lonze and Ginty, 2002). It is thus likely that the memory-constraining actions of PP1 are mediated through inactivation of CREB via dephosphorylation.

Based on *in vitro* studies, it was previously suspected that PP1 activity is attenuated by an endogenous inhibitor named PP1 inhibitor-2 (I-2; Cohen, 1989). However, results by Hou et al. (2013) suggested that the *in vivo* function of I-2 might be more complex than merely inhibiting PP1 activity, because I-2 knock-down led to decreased, rather than increased, PP1 activity in rat primary cortical neurons. A follow-up study by the same group revealed that *in vivo* I-2 indeed enhances rather than suppresses PP1 activity (Yang et al., 2015).

Yang et al. (2015) examined the effects of heterozygous I-2 knock-out in mice and shRNA-mediated knockdown of I-2 in rats. In homozygous I-2 knock-out mice, I-2 was completely absent and these animals died as embryos. Heterozygous animals (I-$2^{+/-}$) on the other hand, were healthy and showed no abnormal phenotypes. I-2 levels in the brains of these animals were ~50% of those detected in wild-type littermates (Yang et al., 2015). I-$2^{+/-}$ mice showed enhanced performance compared with wild types in both novel object recognition and contextual fear conditioning tasks, suggesting that *in vivo*, I-2 limits memory and learning (Yang et al., 2015, their Fig. 1*D*,*E*). To exclude the possibility that these effects were due to unknown developmental compensation, shRNA-mediated-knockdown experiments were performed. The same authors previously showed that such knockdown decreased I-2 levels in cultured cortical neurons (Hou et al., 2013). However, whether the knockdown is similarly efficient *in vivo* has not been shown and data indicating the extent of decreased I-2 expression in the experimental animals is lacking. Nonetheless, a significant positive effect on learning and memory was observed in I-2 knockdown rats in Morris water maze and contextual fear conditioning tasks (Yang et al., 2015, their Fig. 2). These behavioral results suggest that I-2 acts endogenously as a negative regulator of memory function.

The observation that I-2 modulates memory in the same direction as PP1 implies that I-2 has a stimulatory rather than an inhibitory function. In line with this, Yang et al. (2015) found that phosphorylated CREB levels and CREB-mediated gene expression were increased after I-2 reduction in both rats and mice (Yang et al., 2015, their Fig. 3). Further support for an enhancing effect of I-2 on PP1 *in vivo* is

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provided by the finding that PP1 activity was decreased in brain lysates of I-2 $^{+/-}$ mice, whereas PP1 expression levels remained unaffected (Yang et al., 2015, their Fig. 4*A*,*B*). In short, I-2 emerges as a new memory suppressor, potentially acting by enhancing PP1 activity.

Although these findings strongly suggest that I-2's effects on learning and memory task performance are mediated via PP1, it remains possible that I-2 and PP1 independently alter learning and memory performance. To gain conclusive evidence, a follow-up study should contain a comparison between animals in which PP1 or PP1 and I-2 are inhibited. If the effects of I-2 are indeed mediated via PP1, the additional inhibition of I-2 should not cause further effects on learning and memory performance.

Because of the involvement of PP1 and I-2 in learning and memory processes, both might also be implicated in AD, given that this disease is associated with cognitive decline. AD is a neurodegenerative disorder characterized by two types of pathophysiological hallmarks in the brain, namely amyloid plaques and neurofibrillary tangles (Iqbal et al., 2013). Patients suffer from progressively impaired memory function, the severity of which is best correlated with the presence of neurofibrillary tangles (Riley et al., 2002). These neurofibrillary tangles are generated when tau proteins become abnormally (hyper)phosphorylated and oligomerize into paired helical filaments, which subsequently aggregate as tangles (Iqbal et al., 2010). Although it is currently unknown whether I-2 interacts with AD pathology, protein phosphatases have been linked to AD. The activity of PP1 and PP2A (protein phosphatase-2A) is decreased in the brains of AD patients (Gong et al., 1993). Additionally, it has been demonstrated that both PP1 and PP2a can dephosphorylate tau. Although PP2A accounted for 71% of the regulation of tau phosphorylation, PP1 also contributed 11% (Liu et al., 2005). Other protein phosphatases were responsible for the remaining 18%. Interestingly, PP1 was more effective than PP2A in dephosphorylating tau at certain phosphorylation sites (Liu et al., 2005). Therefore, it can be speculated that PP1, together with PP2A, might inhibit the development of AD pathology by decreasing tau hyperphosphorylation and thus tangle formation. It is possible that I-2 has similar inhibitory effects on tau hyperphosphorylation, as it increases PP1 activity (Fig. 1), but no studies have looked into this so far.

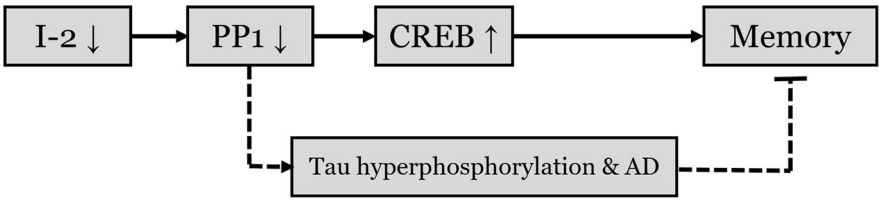

Figure 1. Possible involvement of I-2 and PP1 in learning and memory and Alzheimer's disease. PP1 may affect memory function via two pathways with opposite effects, shown in continuous and dashed lines. Evidence from studies supporting these links is described in the text. I-2: Inhibitor-2; PP1: protein phosphatase-1; CREB: cAMP/calcium response element-binding protein; AD: Alzheimer's disease.

Further support for the inhibitory role of protein phosphatases in AD pathology is given by the observation that when PP2A levels were selectively increased, less tau hyperphosphorylation occurred in transgenic mice expressing a human form of tau (Zhang et al., 2014). Remarkably, these mice also exhibited enhanced cognitive abilities, which possibly resulted from the relieved tau pathology. Furthermore, when PP1 and PP2A were both inactivated in rats with the nonspecific inhibitor calyculin A, tau hyperphosphorylation increased and spatial memory was impaired temporarily (Sun et al., 2003). Studies selectively probing the effect of PP1 on tau hyperphosphorylation and cognition are currently lacking due to absence of selective PP1 inhibitors, and thus no predictions can be made about the possible effects of I-2 on tau pathology.

It must be noted that the results of Sun et al. (2003) seem to contradict those of Yang et al. (2015). Specifically, the combined inhibition of PP1 and PP2A in the study of Sun et al. (2003) resulted in a decrease of memory formation, whereas in the study of Yang et al. (2015) "inhibition" of PP1 alone led to an increase in learning and memory. These apparently contradictory findings could potentially be due to protective effects of PP2A on learning and memory, as observed by Zhang et al. (2014), which may outweigh the inhibitory effects of PP1. Another possible explanation is that PP1 affects cognition via two different pathways with opposing effects. Although PP1 reduces learning and memory via inhibition of CREB, it may slow AD pathology and relieve cognitive impairments by decreasing tau hyperphosphorylation (Fig. 1). Thus, although decreasing PP1 activity by suppressing I-2 is a potential therapeutic intervention to increase memory function, precautions should be taken, as a strong decrease of PP1 activity might result in faster progression of AD pathology.

As it stands, the connection between PP1, I-2, AD, and tau hyperphosphorylation are unclear and warrant further investigation. To study the role of I-2 in the pathophysiology of AD, experiments similar to the one performed by Yang et al. (2015) could be conducted in animal models of tau pathology. Assuming that I-2 is a PP1 stimulator, a decrease in I-2 could lead to more tau hyperphosphorylation, and therefore a faster progression of AD pathology (Fig. 1).

In conclusion, the study of Yang et al. (2015) showed that I-2 stimulates PP1 *in vivo*. These findings are contrary to the previous *in vitro* findings and thus offer a new perspective on the endogenous function of I-2. Furthermore, I-2 likely acts as a memory suppressor through PP1, by stimulating dephosphorylation of CREB and attenuating CREB-dependent gene expression. Downregulating I-2 might thus develop into a possible therapy for cognitive impairments. One application could be AD, as the progression of this disease is associated with memory dysfunction. However, PP1 might affect the pathophysiology of AD via another mechanism, namely via the regulation of tau hyperphosphorylation. Therefore, care should be taken in attempting to alter PP1 activity through I-2, as it might accelerate the progression of AD pathology and the decline of memory functions. Future research should address whether and how the two proposed working mechanisms of PP1 are balanced, and assess whether I-2 can be used as a therapeutic agent without increasing the risk for developing and stimulating AD.

References

Cohen P (1989) The structure and regulation of protein phosphatases. Annu Rev Biochem 58:453–508. CrossRef Medline

Genoux D, Haditsch U, Knobloch M, Michalon A, Storm D, Mansuy IM (2002) Protein phosphatase 1 is a molecular constraint on learning and memory. Nature 418:970–975. CrossRef Medline

Gong CX, Singh TJ, Grundke-Iqbal I, Iqbal K (1993) Phosphoprotein phosphatase activities in Alzheimer disease brain. J Neurochem 61:921–927. CrossRef Medline

Hou H, Sun L, Siddoway BA, Petralia RS, Yang H, Gu H, Nairn AC, Xia H (2013) Synaptic NMDA receptor stimulation activates PP1 by inhibiting its phosphorylation by Cdk5. J Cell Biol 203:521–535. CrossRef Medline

Iqbal K, Liu F, Gong CX, Grundke-Iqbal I (2010) Tau in Alzheimer disease and related tauopathies. Curr Alzheimer Res 7:656–664. CrossRef Medline

Iqbal K, Flory M, Soininen H (2013) Clinical symptoms and symptom signatures of Alzheimer's disease subgroups. J Alzheimers Dis 37: 475–481. CrossRef Medline

Liu F, Grundke-Iqbal I, Iqbal K, Gong CX (2005) Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. Eur J Neurosci 22:1942–1950. CrossRef Medline

Lonze BE, Ginty DD (2002) Function and regulation of CREB family transcription factors in the nervous system. Neuron 35:605–623. CrossRef Medline

Riley KP, Snowdon DA, Markesbery WR (2002) Alzheimer's neurofibrillary pathology and the spectrum of cognitive function: findings from the Nun Study. Ann Neurol 51:567–577. CrossRef Medline

Sun L, Liu SY, Zhou XW, Wang XC, Liu R, Wang Q, Wang JZ (2003) Inhibition of protein phosphatase 2A- and protein phosphatase 1-induced tau hyperphosphorylation and impairment of spatial memory retention in rats. Neuroscience 118:1175–1182. CrossRef Medline

World Health Organization (2015) Fact sheet N° 362: dementia. http://www.who.int/mediacentre/factsheets/fs362/en/ (Accessed on: 17-12-2015).

Yang H, Hou H, Pahng A, Gu H, Nairn AC, Tang YP, Colombo PJ, Xia H (2015) Protein phosphatase-1 inhibitor-2 is a novel memory suppressor. J Neurosci 35:15082–15087. CrossRef Medline

Zhang Y, Ma RH, Li XC, Zhang JY, Shi HR, Wei W, Luo DJ, Wang Q, Wang JZ, Liu GP (2014) Silencing I2PP2A recues tau pathologies and memory deficits through rescuing PP2A and inhibiting GSK-3β signaling in human tau transgenic mice. Front Aging Neurosci 6:123. CrossRef Medline